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## SHORT COMMUNICATION

### Susceptibility of apple clearwing moth larvae, *Synanthedon myopaeformis* (Lepidoptera: Sesiidae) to *Beauveria bassiana* and *Metarhizium brunneum*

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Apple clearwing moth larvae, *Synanthedon myopaeformis* (Lepidoptera: Sesiidae) were found to be susceptible to infection by two entomopathogenic fungi: an indigenous fungus isolated from *S. myopaeformis* cadavers and identified as *Metarhizium brunneum* (Petch); and *Beauveria bassiana* isolate GHA. In laboratory bioassays, larvae exhibited dose related mortality after exposure to both the *M. brunneum* and *Beauveria bassiana* with 7 day LC<sub>50</sub>'s of  $2.9 \times 10^5$  and  $3.4 \times 10^5$  spores/mL, respectively. Larval mortalities caused by the two isolates at  $1 \times 10^6$  spores/mL were not significantly different and 73% of the *M. brunneum*-treated, and 76% of the *B. bassiana*-treated larvae were dead 7 days post treatment, with LT<sub>50</sub>'s of 5.5 and 5.1 days, respectively.

**Keywords:** *Synanthedon myopaeformis*; *Beauveria bassiana*; *Metarhizium brunneum*; entomopathogenic fungi; biological control

The apple clearwing moth (ACWM), *Synanthedon myopaeformis* (Borkhausen) (Lepidoptera: Sesiidae), was found for the first time in southern British Columbian (BC) apple orchards in 2005 (Judd and Philip 2006). The moths of this invasive European species emerge from June through August in BC, ovipositing on damaged bark near graft unions and pruning cuts. The emerging larvae bore into the tree to feed within tunnels, primarily at the base of the tree (Ateyyat 2006). Larval development extends over a possible 2-year period (G. Judd, personal observation). *Synanthedon myopaeformis* infestations in Europe have been recorded as damaging to both the health of the trees and their yield (Dickler 1977; Castellari 1987). Control strategies for the species in Eurasia have included mass trapping of the adults (Trematerra 1993), mating disruption (Steuber and Dickler 1987; Kyparissoudas and Tsourgianni 1993), insecticide treatments (Castellari 1987; Ateyyat 2005) and coating or wrapping the damaged bark (Ciglar and Masten 1979; Van Frankenhuyzen and Wijnen 1979; Kilic, Aykac, and Cevik 1988; Ateyyat and Antary 2006). Nematodes and *Bacillus thuringiensis* Berliner are the only recorded entomopathogenic agents assessed against *S. myopaeformis* (Deseo and Miller 1985; Kahounova and Mracek 1991; Shehata, Nasr, and Tadros 1999). Several studies have recorded field collected

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current clearwing moth larvae, *Synanthedon tipuliformis* (Clerck), to be infected by entomopathogenic fungi, including *Beauveria bassiana* (Balsamo) Vuillemin (Scott and Harrison 1979; Baker 1981; Hardy 1982), however there is no record of the potential of fungi to suppress any *Synanthedon* species.

Several *S. myopaeformis* larvae collected from apple orchards in Cawston, BC were found to host an unknown fungus. The objectives of this study were to identify the indigenous pathogen and determine its pathogenicity, in comparison to that of *B. bassiana* isolate GHA, to *S. myopaeformis* larvae under laboratory conditions.

Spores from orchard-collected ACWM cadavers were plated on Potato Dextrose Agar (PDA) and incubated at 25°C. Isolates were identified initially according to morphological characters (Humber 1997; Bischoff, Rehner, and Humber 2009), and identifications were confirmed by sequencing the internally transcribed spacer regions (ITS) of the ribosomal DNA repeat (ITS1 and 2) and the intron-rich region at the 5' end of the transcription elongation factor 1- $\alpha$  (tef) gene (eEF1a1). Amplification and sequencing was performed using the primers and protocols described by Shoukouhi and Bissett (2009) and Hoyos-Carvajal, Orduz, and Bissett (2009). The tef sequence obtained using these techniques was unambiguous over the entire sequenced region of more than 750 nucleotides. It differed by only 5 nt from Genbank accession EU248855, identified as *M. brunneum* Petch (Bischoff et al. 2009). The ACWM-isolate clustered with all of the *M. brunneum* isolates with sequences deposited in Genbank and was in agreement with the morphological description of the species by Bischoff et al. (2009). The isolate has been deposited in the Canadian Collection of Fungal Cultures (K.W. Neatby Bldg., Ottawa, Ontario, Canada) and ITS and tef sequences deposited in Genbank.

Both the ACWM isolate of *M. brunneum* and the *B. bassiana*-GHA (LRC 26 from AAFC collection, Lethbridge, AB) were cultured on PDA for at least 2 weeks before use in larval bioassays. For each assay, plates were washed with sterile dH<sub>2</sub>O and the concentration of each suspension was determined using a haemocytometer. Dilutions were prepared with sterile dH<sub>2</sub>O.

Larvae for the bioassays were obtained from ACWM-infested apple, *Malus domestica* Borkhausen, tree trunks that were cut and removed from an orchard in Cawston, BC prior to winter and stored in an outdoor screened cage over a 4-month period when air temperatures ranged from -21.0 to 16.0°C. One day before each laboratory trial, chisels were used to expose larvae in their feeding tunnels and 120 to 150 larvae were collected with forceps. The larvae were held on damp paper towels at 15°C for 20–24 h and each larva was washed with a 5% bleach solution and rinsed twice in sterile distilled water before use. Larval head capsules were measured using an ocular micrometer on a stereo microscope. Only larger larvae with head capsules equal to, or exceeding 1 mm were used in this study and larvae were evenly distributed across treatments based on size.

On the day of, or day before each assay, young apple tree branches (approximately 1 cm diameter) were cut from trees in an orchard that was not treated with chemical insecticides. Each branch was wiped with 70% ethanol and then sterile dH<sub>2</sub>O before it was cut into approximately 1 cm lengths. Each piece was subsequently split in half lengthwise using flame sterilized pruners. A single piece of split apple branch was placed bark down on 5 to 10 mm of agar poured and cooled in a 30-mL plastic cup. Each of 20 larvae within each treatment was submerged in 20 mL of a fungal suspension, or the control, for approximately 5 s before superficial

liquid was drained on a clean paper towel and each treated larva was placed individually on top of a split piece of apple branch. To evaluate the dose-dependent efficacy of each fungal isolate larvae were exposed to one of four fungal concentrations ( $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$  and  $1 \times 10^7$  spores/mL) or the control of sterile dH<sub>2</sub>O. Each cup containing an inoculated larva on a piece of apple wood on agar, was sealed with a lid and all cups incubated at 25°C under a 16 h L:8 h D photoperiod. Mortality and mycosis were recorded every 24 h. Each bioassay was replicated three times over days. The large number of larvae required per dose dependent assay and the time required to extract the larvae from the infested wood limited the tests to only one fungal isolate per day.

To compare the efficacy of the two fungi in the same trial, the spores of *B. bassiana*-GHA and the indigenous ACWM isolate were washed from PDA plates and a single concentration of  $1 \times 10^6$  spores/mL dH<sub>2</sub>O prepared as described above. Sterile dH<sub>2</sub>O was used as the control. Each of 15 ACWM larvae was submerged in each treatment or control and mortality and mycosis were recorded as described above. The trials were replicated three times over days.

Abbott's formula was used to correct the percent mortality before probit analysis to determine median lethal times and concentrations with 95% confidence intervals using PROBIT (SAS 2004). Percent mortalities in the comparison bioassays were arcsine transformed before being subjected to an ANOVA (SAS 2004). Means were compared using Tukey's studentized range test ( $\alpha = 0.05$ ).

The *S. myopaeformis* larvae were susceptible to infection and subsequent dose related mortality by both fungi. The median lethal concentration (LC<sub>50</sub>) of the *M. brunneum*-ACWM at 7 days post-treatment was  $2.9 \times 10^5$  spores/mL (95% CI:  $1.3 \times 10^5 - 6.4 \times 10^5$  spores/mL). The LC<sub>50</sub> of the *B. bassiana* GHA isolate was similar at  $3.4 \times 10^5$  spores/mL (95% CI:  $1.5 \times 10^5 - 6.7 \times 10^5$  spores/mL). Mortality and mycosis began for both isolates on day two. The median time to cause mortality, (LT<sub>50</sub>) for the *M. brunneum*-ACWM at  $1 \times 10^6$  spores/mL was 5.5 days (CI: 4.7–6.8 days). The LT<sub>50</sub> for the *B. bassiana* isolate was similar at 5.1 days (CI: 4.4–6.1 days).

In the comparison bioassay, mortalities caused by the two isolates at  $1 \times 10^6$  spores/mL were not significantly ( $P > 0.05$ ) different from one another, however both were significantly higher than the control (day 7,  $F_{2,4} = 53.30$ ,  $P = 0.001$ ; mean  $\pm$  SE control mortality =  $6.7 \pm 3.9\%$ ). *B. bassiana* and *M. brunneum* at  $1 \times 10^6$  spores/mL caused mean  $\pm$  SE mortalities of  $75.6 \pm 4.4$  and  $73.3 \pm 3.9\%$ , 7 days post treatment and  $91.1 \pm 5.9$  and  $91.1 \pm 2.2\%$  mortality 14 days post-treatment, respectively. It is unfortunate that due to the time required to extract test insects from the infested wood it was not possible to run the multiple dose bioassays for the two fungal isolate in parallel, however the results of the comparative bioassay support the results from the individual assays and thus it is likely that differing parameters during the latter were minimal.

Based on these data, both *M. brunneum*-ACWM and *B. bassiana*-GHA isolates are good candidates for further study as biological control options to suppress ACWM larvae under field conditions. *M. brunneum* has been recorded to infect other Lepidoptera including spotted stalk borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and white stem borer *Scirpophaga innotata* (Walker) (Lepidoptera: Pyralidae) in laboratory trials (Gardezi 2006). *Metarhizium* spp. are frequently isolated from soils (Bischoff et al. 2009) and we cannot be certain that the infected *S. myopaeformis* larvae naturally encountered the *M. brunneum* isolate or whether

they were contaminated through contact with soil while extracting the larvae. *B. bassiana* is a known endophyte (Vega et al. 2008) and may be compatible with larval suppression within the *S. myopaeformis* tunnels.

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